*ENZYMATIC BREAKAGE AND JOINING OF DEOXYRIBONUCLEIC ACID, I. REPAIR OF SINGLE-STRAND BREAKS IN DNA BY AN ENZYME SYSTEM FROM ESCHERICHIA COLI INFECTED WITH T4 BACTERIOPHAGE**

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Enzymatic breakage and joining of preformed polynucleotide strands has been implicated in molecular recombination, the repair of ultraviolet-irradiated DNA, and the interconversion of linear and circular DNA molecules.[1] A possible intermediate, in each of these processes, is a duplex DNA molecule containing phosphodiester bond interruptions (single-strand breaks). This paper describes the purification and some properties of an enzyme system, polynucleotide ligase, which catalyzes the repair of single-strand breaks by the formation of phosphodiester bonds (Fig. 1). The enzyme system has been purified from *Escherichia coli* infected with T4 bacteriophage and found to require ATP for activity.

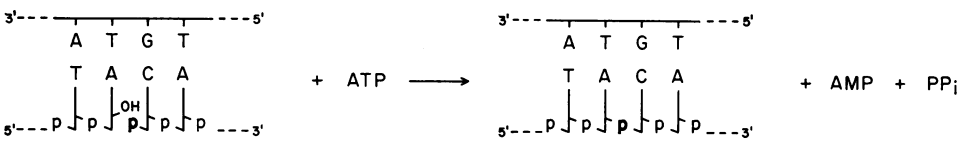

FIG. 1.—Polynucleotide ligase catalyzes the formation of a phosphodiester bond at the site of a single-strand break in a duplex DNA molecule, resulting in the covalent joining of two polynucleotides.

In order to isolate and to characterize the enzyme system, procedures have been developed (1) for the preparation and characterization of T7 DNA containing single-strand interruptions, (2) for distinguishing external 5′-phosphomonoesters from those located internally in a duplex molecule, and (3) for the removal of internal 5′-phosphomonoesters and their replacement with P^{32}-labeled groups. The latter procedure enables the synthesis of a unique substrate for the detection of enzymes which join polynucleotides.

Experimental Procedure.—Materials: Unlabeled and radioactively labeled nucleotides, salmon sperm DNA, and T7 phage DNA were obtained as previously described.[2] The purified enzymes were the same preparations previously described.[3] Spleen phosphodiesterase and pancreatic DNase (2000 units per standard vial) were products of the Worthington Biochemical Co. Concentrations of DNA are expressed as equivalents of nucleotide phosphorus.

Methods: (a) *Preparation of T7 DNA containing single-strand breaks (nicked DNA):* Pancreatic DNase is known to produce single-strand breaks in native DNA.[4, 5] The incubation mixture (12 ml) contained 1.3 mM T7 DNA, 67 mM Tris buffer (pH 8.0), 5 mM $MgCl_2$, and 0.17 unit of pancreatic DNase. The enzyme was diluted, immediately before use, into a solution containing 10 mM sodium acetate buffer (pH 5.5), 5 mM $MgCl_2$, 0.2 *M* NaCl, and bovine plasma albumin (0.5 mg per ml) to a concentration of 0.21 unit per ml. After incubation at 20° for 30 min, 0.4 ml of 0.5 *M* Na-EDTA (pH 8.0) was added, and the mixture was dialyzed against 0.01 *M* Tris buffer (pH 8.0)–0.02 *M* NaCl. This DNA, containing an average of eight single-strand breaks per strand (see section *e*), will be referred to as nicked DNA (Fig. 2*b*).

(b) *Preparation of T7 DNA containing internal P^{32}-phosphomonoesters:* Pancreatic DNase cleaves phosphodiester bonds so as to produce 5′-phosphoryl end groups.[6] These internal phos-

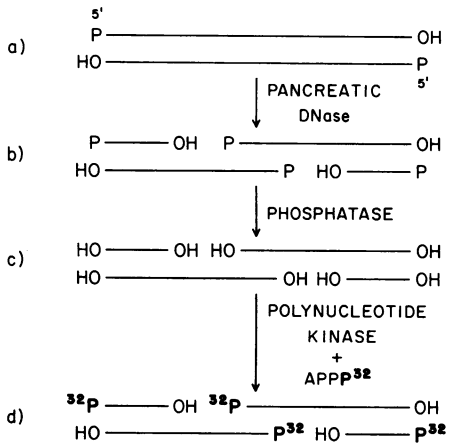

FIG. 2.—Scheme for the preparation of 5′-P^{32}-phosphoryl nicked DNA, substrate for polynucleotide ligase. The two strands of a duplex T7 DNA molecule are schematically represented by two parallel lines; the 5′-termini of each have been previously shown to bear phosphoryl groups.[2]

phomonoesters can be removed by incubation at elevated temperatures with alkaline phosphatase from *E. coli*. The incubation mixture (5.1 ml) contained 1.2 mM nicked DNA, 0.01 *M* Tris buffer (pH 8.0), 0.02 *M* NaCl, and 7 units of phosphatase. Incubation was at 65° for 30 min; additional enzyme (7 units) was added at 10 and 20 min. End-group analysis of the DNA revealed that 95% of the phosphomonoesters had been removed (Fig. 2*c*). These groups were replaced by P^{32}-phosphomonoesters by incubation with ATP^{32} in the polynucleotide kinase reaction.[7] The phosphatase incubation mixture was made 28 mM with Tris buffer (pH 8.0), 10 mM with $MgCl_2$, 17 mM with 2-mercaptoethanol, and 14 mM with potassium phosphate buffer (pH 7.5);[8] water was added to a final volume of 9 ml. After mixing, 0.17 μmoles of ATP^{32} (9×10^9 cpm per μmole) and 500 units of polynucleotide kinase were added. Incubation was at 37° for 45 min with an additional 200 units of kinase added at 15 and 30 min.

The labeled nicked DNA was isolated by fractionation on a column of methylated albumin-Kieselguhr (20 ml of MAK in a column 3 cm in diameter).[9] A 6-ml portion of the phosphatase reaction mixture was diluted tenfold with 0.15 *M* NaCl–0.015 *M* sodium citrate and applied to the column. The adsorbent was washed with 100 ml of the above solution, followed by 200 ml of 0.05 *M* Tris buffer (pH 6.7)–0.4 *M* NaCl, and the DNA was eluted with 0.05 *M* Tris buffer (pH 6.7)–0.95 *M* NaCl. The eluate (25 ml) was dialyzed against 0.01 *M* Tris buffer (pH 7.9)–0.05 *M* NaCl.

The final preparation (3.7 μmoles of DNA in 30 ml) contained six 5′-P^{32}-phosphoryl end groups per original strand of T7 DNA (see below). Of these, 60% were located at the sites of single-strand interruptions. This DNA preparation will be referred to as 5′-P^{32}-phosphoryl nicked DNA (Fig. 2*d*), and details of its full characterization will be described separately.

(c) *Assay of polynucleotide ligase:* The standard assay measures the conversion of 5′-P^{32}-phosphomonoesters in nicked DNA into a form which remains acid-insoluble after incubation with phosphatase. Polynucleotide ligase was routinely diluted into a solution containing 0.05 *M* Tris buffer (pH 7.6), 0.01 *M* 2-mercaptoethanol, and bovine plasma albumin (0.5 mg per ml). The incubation mixture (0.3 ml) contained 0.02 mM 5′-P^{32}-phosphoryl nicked DNA, 0.066 mM ATP, 33 mM Tris buffer (pH 7.6), 6.6 mM $MgCl_2$, 6.6 mM 2-mercaptoethanol and 5×10^{-5} to 3×10^{-4} unit of enzyme. After incubation at 37° for 20 min, 0.2 ml of salmon sperm DNA (0.25 mg per ml), 0.5 ml of cold 0.6 *M* trichloroacetic acid, and 2.0 ml of cold water were added in succession. After centrifugation at 10,000 × *g* for 10 min, the supernatant fluid was discarded. Two ml of cold 0.01 *M* HCl were added to the tube, mixed, and recentrifuged, and the supernatant fluid was discarded. The precipitate was dissolved in 0.5 ml of 0.1 *M* NaOH and neutralized by adding 0.05 ml of 1.1 *M* HCl–0.2 *M* Tris. Alkaline phosphatase (10 μg) was added, and each reaction mixture was incubated for 15 min at 37°, followed by 15 min at 65°; then 0.5 ml of cold 0.6 *M* trichloroacetic acid and 2.0 ml of cold water were added. The precipitate was collected on glass filters, washed, and dried, and the radioactivity determined as previously described.[7]

The precipitate obtained from control incubations with ligase omitted contained 5% of the added radioactivity. The precipitate obtained from control incubations with both enzyme and phos-

TABLE 1

PURIFICATION OF POLYNUCLEOTIDE LIGASE

Fraction	Total activity (units)	Specific activity (units/mg)
I. Extract	1040	2.2
II. Streptomycin	450	1.0
III. Ammonium sulfate	450	3.9
IV. DEAE-cellulose	360	3.8
V. DEAE-cellulose	180	23
VI. Phosphocellulose	95	770

phatase omitted contained 95% of the added radioactivity. One unit of the enzyme is defined as the amount catalyzing the conversion of 1 mμmole of P^{32}-phosphomonoesters into a phosphatase-resistant form in 20 min. The activity was proportional to enzyme concentration in the range given above.

(d) *Large-scale preparation of DNA product of ligase reaction:* For characterization of the DNA product of the reaction, 1.2 μmoles of 5′-P^{32}-phosphoryl nicked DNA was incubated in a reaction mixture (3 ml) containing 0.03 units of fraction VI and the same concentrations of the other components of the standard reaction mixture. Incubation was for 60 min at 37°. The reaction mixture was dialyzed extensively to remove ATP as previously described.[2] Recovery of radioactivity and of DNA was 94%.

DNA containing only phosphatase-resistant P^{32} was prepared by incubating the product of the ligase reaction with phosphatase at 65°. The phosphatase was removed by phenol extraction.[2] After denaturation of the DNA product, 97% of the P^{32} was found to be resistant to phosphatase.

(e) *End-group analysis of DNA:* The number of 5′-termini in a DNA preparation was determined, after denaturation and dephosphorylation of the DNA, by radioactive labeling of these end groups in the polynucleotide kinase reaction.[2, 3, 7] The number of external 5′-termini in nicked DNA was measured by end-group labeling after treatment of the helical DNA with phosphatase at 37°. Under appropriate conditions, at 37°, phosphatase will hydrolyze only external phosphomonoesters (less than 5% hydrolysis of internal phosphomonoesters). The number of internal 5′-termini was determined by subtracting the number of external 5′-termini from the total number of 5′-termini, determined as described above. The location of P^{32}-labeled phosphomonoesters in DNA preparations was determined in a similar manner. A detailed description of the action of *E. coli* alkaline phosphatase on external and internal phosphomonoesters will be the subject of a separate paper.

(f) *Other methods:* The following methods have been described previously: measurements of protein, deoxypentose, and phosphate; hydrolysis of DNA to either 5′- or 3′-mononucleotides;[2, 10] and electrophoretic separation of nucleotides and PP_i.[2]

(g) *Purification of polynucleotide ligase:* All operations were performed at 4°, centrifugations were for 10 min at 10,000 × *g*, and all solutions contained 0.01 *M* 2-mercaptoethanol. The results of a typical preparation are summarized in Table 1.

Preparation of extracts: T4r$^+$ phage-infected *E. coli* cells (5 gm) were grown, collected, and disrupted, and the cell debris was removed as previously described.[7] The final supernatant fluid was diluted with buffer to an optical density at 260 mμ of 68 (fraction I).

Streptomycin: To 70 ml of extract were added, with stirring, 14 ml of 5% streptomycin sulfate over a 30-min period. The suspension was centrifuged and the supernatant fluid was collected (fraction II).

Ammonium sulfate fractionation: To 77 ml of fraction II, 25.4 gm of ammonium sulfate were added, with stirring, over a 30-min period. The precipitate was collected by centrifugation and dissolved in 10 ml of 0.01 *M* Tris buffer (pH 7.6)–0.1 *M* NaCl (fraction III).

DEAE-cellulose fractionation: A column of DEAE-cellulose (4 cm^2 × 12 cm) was washed with 1.5 liters of 0.01 *M* Tris buffer (pH 7.6). Fraction III (135 mg of protein) was dialyzed against 2 liters of this buffer for 12 hr, and applied to the column. The adsorbent was washed with 100 ml of the buffer, and the enzyme was eluted with 0.01 *M* Tris buffer (pH 7.6)–0.3 *M* NaCl. Approximately 70% of the activity applied to the adsorbent was obtained in 50 ml of the eluate (fraction IV).

DEAE-cellulose chromatography: A column of DEAE-cellulose (4 cm^2 × 12 cm) was prepared and washed as before. Fraction IV (100 mg of protein) was dialyzed for 12 hr against 2 liters of

the washing buffer, applied to the column, and the adsorbent was washed with 100 ml of the same buffer. A linear gradient of elution (total volume of 900 ml) was applied with 0.01 *M* Tris buffer (pH 7.6) and 0.01 *M* Tris buffer (pH 7.6)–0.3 *M* NaCl as limiting concentrations. Of the activity applied, 80% was eluted between 6.6 and 8.7 resin bed volumes of effluent. The fractions containing enzyme of specific activity greater than 20 units per mg of protein were pooled (fraction V).

Phosphocellulose fractionation: A column of phosphocellulose (1 cm^2 × 11 cm) was prepared and washed with 1 liter of 0.01 *M* potassium phosphate buffer (pH 7.6)–0.1 *M* KCl. Sixty ml of fraction V were diluted to a volume of 110 ml with 0.01 *M* potassium phosphate buffer (pH 7.6) and then applied to the column. The adsorbent was washed with 25-ml portions of 0.01 *M* potassium phosphate buffer (pH 7.6) containing the following concentrations of KCl: 0.1 *M*, 0.25 *M*, of 0.5 *M*. Of the activity applied, 75% was obtained in 10 ml of the 0.5 *M* KCl eluate (fraction VI).

The phosphocellulose fraction was purified 350-fold over the extract and contained 10% of the activity initially present. Preparations of fraction VI have lost 50% of their activity during 2 weeks' storage in an ice bath.

Results.—Appearance of polynucleotide ligase activity after infection with phage T4: Polynucleotide ligase, measured by the standard assay, could be detected in extracts of *E. coli* about five minutes after infection with T4 phage. A maximal level of activity (2.2 units per mg of protein) was observed approximately 20 minutes after infection. Extracts of uninfected *E. coli* contained no detectable activity (less than 0.1 unit per mg of protein).

Properties of the purified enzyme: With fraction VI the activity in the standard assay was dependent upon the addition of ATP and Mg^{++} (Table 2). A sulfhydryl

TABLE 2

REQUIREMENTS FOR LIGASE ACTIVITY

Components	P^{32} resistant to phosphate ($\mu\mu$moles)
Complete system	0.20
Minus nicked DNA	<0.01
Minus ATP	<0.01
Minus Mg^{++}	<0.01
Minus 2-mercaptoethanol	0.12
Minus enzyme	<0.01

Conditions of the standard assay were employed with 2 × 10^{-4} unit of fraction VI in each tube. In testing the omission of 2-mercaptoethanol from the reaction mixture, the enzyme dilution was made in the absence of this compound.

compound was required for optimal activity. dATP, dTTP, GTP, or ADP could not replace ATP in the reaction mixture. The optimal pH range for the reaction is 7.5–8.0 in Tris buffer. During incubation in the standard assay, the purified enzyme (0.02 unit) released no acid-soluble radioactivity (less than 5%) from helical or denatured 5′-P^{32}-phosphoryl nicked DNA substrate, either in the presence or absence of ATP. There was no detectable hydrolysis of ATP^{32} (less than 1%) during a 30-minute incubation with 0.5 unit of fraction VI.

Reaction occurs at site of single-strand breaks: (1) *Requirement of single-strand breaks:* With the addition of excess ligase or with prolonged incubation, the amount of P^{32} resistant to phosphatase reached a limit. When an additional equal amount of DNA substrate was added to the reaction mixture, a further reaction occurred, equal in extent to that initially observed.

However, not all of the P^{32}-phosphomonoesters in the nicked DNA substrate were rendered resistant to phosphatase during prolonged incubation in the ligase reaction. As shown in Table 3, the limit of the reaction could be correlated closely with the number of internal P^{32}-phosphomonoesters present in a substrate.

TABLE 3

REQUIREMENT FOR SINGLE-STRAND BREAKS

DNA	P^{32} at single-strand breaks (%)	P^{32} made resistant to phosphatase (%)
Native DNA	<5	<5
Nicked DNA		
Externally labeled	<12	<5
Externally and internally labeled	50	50
Internally labeled	85	90
Denatured, nicked DNA	<5	<5

Each DNA preparation was tested in the standard assay. The percentage of total P^{32} made resistant to phosphatase represents the limit reached in a 30-min incubation with the addition of 0.02 unit of fraction VI at 0 and 15 min. The terms externally and internally labeled refer to the presence of external and internal 5′-P^{32}-phosphomonoesters, respectively. The DNA's were prepared and the amount of P^{32}-phosphomonoesters located at single-strand breaks in each was determined as described in *Methods*.

Samples of DNA's containing different ratios of internal to external phosphomonoesters were incubated in reaction mixtures containing an excess of enzyme. Native or nicked T7 DNA bearing only external P^{32}-phosphomonoesters did not serve as substrates. However, nicked DNA preparations in which 50 and 85 per cent of the P^{32}-phosphomonoesters were internally located were substrates; 50 and 90 per cent, respectively, of their total P^{32} became resistant to phosphatase. Denaturation of DNA preparations containing internal P^{32}-phosphomonoesters destroyed their ability to function as substrates.

(2) *Identification of P^{32}-phosphomonoesters after the ligase reaction:* Evidence that the reaction involved only internal phosphomonoesters was also obtained by determining the location of the unreacted phosphomonoesters in the DNA product (Table 4). All of the original external P^{32}-phosphomonoesters were still present as such; but 95 per cent of those phosphomonoesters initially present at single-strand breaks had become resistant to phosphatase.

Characterization of the DNA product: (1) *3′, 5′-Phosphodiester bond formation at sites of joining.* A product of the ligase reaction, containing only phosphatase-resistant P^{32} (see *Methods*), was hydrolyzed with pancreatic DNase and snake venom phosphodiesterase. More than 95 per cent of the radioactivity was recovered as nucleoside 5′-monophosphates. When the sample was hydrolyzed with micrococcal and spleen nucleases, more than 95 per cent of the radioactivity was identified as nucleoside 3′-monophosphates. Confirmation of the presence of P^{32} in 3′,5′-phosphodiester bonds was obtained by hydrolyzing the DNA with *E. coli* exonuclease I. All of the P^{32} (more than 90%) was recovered as 5′-mononucleotides. (Exonuclease I is unable to hydrolyze the phosphodiester bond of a dinucleotide and has been used previously to determine the location of P^{32} in a polynucleotide.)[7]

TABLE 4

IDENTIFICATION OF P^{32}-PHOSPHOMONOESTERS AFTER LIGASE REACTION

DNA	External P^{32}-monoesters	Internal P^{32}-monoesters (percentage of total P^{32})	Phosphatase-insensitive P^{32}
Nicked DNA substrate	33	67	<1
Product	35	3	62

The percentage of external and internal P^{32}-phosphomonoesters and of phosphatase-resistant P^{32} in the substrate and product were determined as described in *Methods*. The nicked DNA substrate, containing internal and external P^{32}-phosphomonoesters, and the product of the ligase reaction were prepared as described in *Methods*.

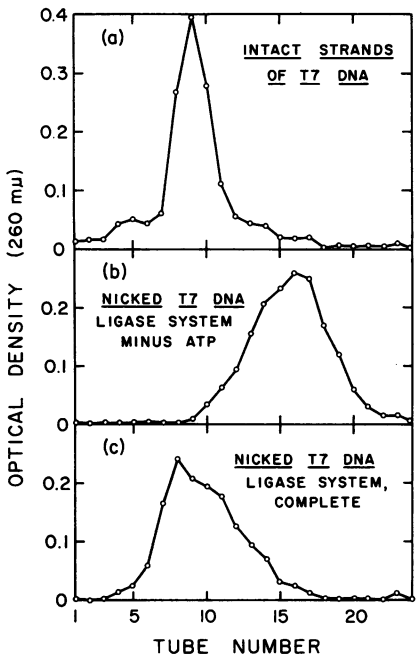

FIG. 3.—Analysis of the DNA product of the ligase reaction by preparative zone sedimentation in alkaline sucrose gradients. T7 DNA was sedimented prior to any enzymatic incubations (*a*), after treatment with pancreatic DNase (nicked DNA) followed by incubation in the ligase reaction from which ATP was omitted (*b*), and after treatment with pancreatic DNase followed by incubation in the complete ligase reaction (*c*). All DNA preparations were denatured prior to sedimentation.

Nicked T7 DNA (400 mμmoles) was prepared as described in *Methods* and incubated for 60 min in the ligase reaction (0.6 ml) with 0.15 unit of fraction VI. A control incubation contained no ATP. The reaction mixtures were chilled, and 0.025 ml of 0.5 M sodium EDTA (pH 8.0) was added. After dialysis for 18 hr at 4° against 0.01 *M* Tris buffer (pH 7.4)–0.05 *M* NaCl, the DNA was denatured and 300 mμmoles were analyzed by zone sedimentation in alkali as previously described.[2]

(2) *Demonstration, by sedimentation analysis, that polynucleotides are joined:* Zone sedimentation of DNA in alkali can provide information about the molecular weight of single polynucleotide strands.[11] Denatured T7 DNA, prior to any enzymatic treatment, sedimented as a single component (Fig. 3*a*). By contrast, denatured, nicked DNA sedimented at a greatly reduced rate; no intact single strands were found in the preparation. When the helical nicked DNA was incubated in the ligase reaction, at least 80 per cent of the fragmented DNA increased in sedimentation rate, and approximately 50 per cent acquired sedimentation properties indistinguishable from those of intact strands of T7 DNA (Fig. 3*c*). In a control experiment helical, nicked DNA was incubated in the ligase reaction in the *absence* of ATP and then analyzed in a similar manner (Fig. 3*b*). After incubation, this DNA had sedimentation properties identical to those of the untreated nicked DNA.

Zone sedimentation of these DNA preparations at neutral pH, without prior denaturation, revealed that they all had the same sedimentation properties. This finding indicated that no end-to-end joining of duplex molecules had occurred and confirmed the results described in Table 3.

(3) *Decrease in 5′-termini of DNA during ligase reaction:* End-group analysis of the nicked DNA and the DNA product of the polynucleotide ligase reaction provided an additional means for demonstrating the joining of polynucleotides. A preparation of nicked T7 DNA contained 1.7 external and 7.1 internal 5′-termini per original intact strand. After incubation in the ligase reaction (conditions of incubation and isolation of DNA as in Fig. 3), the DNA product still contained 1.7 external 5′-termini but only 1.6 internal 5′-termini per strand.

AMP and PP_i are products of the reaction: In order to identify the reaction products shown in Figure 1, T7 DNA was incubated with polynucleotide ligase in

TABLE 5

IDENTIFICATION OF AMP AND PP_i AS PRODUCTS OF THE LIGASE REACTION

Time (min)	ATP (mμ moles)	AMP (mμ moles)	PP_i (mμ moles)
0	10.4	<0.1	<0.1
30	7.2	3.4	3.4
Δ	−3.2	+3.4	+3.4

T7 DNA was incubated in a ligase reaction with pancreatic DNase. The reaction mixture (0.3 ml) contained 0.33 mM T7 DNA, 0.034 mM ATP (H^3-ATP and γ-P^{32}-ATP), 6.6 mM $MgCl_2$, 6.6 mM 2-mercaptoethanol, 33 mM Tris buffer (pH 7.6), 264 mM KCl, 10 μg of bovine plasma albumin, 0.18 units of pancreatic DNase, and 0.5 units of polynucleotide ligase (fraction VI). After 30 min of incubation at 37°, a 0.02-ml sample was removed and analyzed for AMP, ADP, ATP, and PP_i by electrophoresis at pH 5 (see *Methods*). Recovery of radioactivity was greater than 95%. PP_i was further identified by its failure to adsorb to Norit and to complex with molybdate[12] unless first treated with yeast pyrophosphatase. When DNA, pancreatic DNase, or the ligase was omitted, less than 1% of the ATP was consumed. No ADP (less than 1% of AMP formed) was detected in the reaction mixture, after 30 min of incubation.

the presence of pancreatic DNase. This permitted recycling of DNA in alternate hydrolysis and repair reactions, with a large net hydrolysis of ATP. The disappearance of ATP from the reaction mixture paralleled the appearance of equal amounts of AMP and PP_i (Table 5). This reaction was completely dependent on the presence of DNA, DNase, and polynucleotide ligase.

Discussion.—T4 phage infection of *E. coli* induces an enzyme system which catalyzes the repair of single-strand breaks in a DNA duplex by the formation of phosphodiester bonds; ATP and Mg^{++} are required for the reaction. The observed cleavage of ATP to AMP and PP_i suggests two possible mechanisms for the formation of these phosphodiester bonds. (1) PP_i could be transferred from ATP to a 5′-phosphoryl terminus at a single-strand break, to form AMP and an activated 5′-terminus bearing a triphosphate. Condensation of the polynucleotides would release PP_i. (2) Alternatively, activation could result from the formation of a 5′-phosphoryl DNA adenylate intermediate (ADP-DNA), releasing PP_i. In this case the formation of phosphodiester bonds would eliminate AMP. Although the ligase system has been purified 350-fold, it is not known if more than one enzyme is involved in the over-all reaction. However, it appears that this enzyme system, by activating a polynucleotide, provides a mechanism for the formation of phosphodiester bonds which differs from those usually invoked for DNA synthesis.[13] (See *Note added in proof.*)

The function of this enzyme system *in vivo* is not known, but the molecular events which have been observed after T-even phage infection of *E. coli* suggest the following possible role. After infection, parental phage DNA undergoes extensive fragmentation and dispersion among other parental and progeny molecules.[14–16] These recombinant molecules contain single-strand interruptions which are eventually repaired, so that the recombined fragments are covalently joined. It is possible that polynucleotide ligase is involved in this last step. Although polynucleotide ligase activity has been identified only in cells infected with T4 phage, it is possible that it exists in greatly reduced amounts in uninfected cells. For example, Gellert[17] has recently described an activity in extracts of several *E. coli* strains which converts hydrogen-bonded circles of λ DNA to covalent circles.

Other *in vitro* studies on molecular recombination and DNA repair will be greatly facilitated by the availability of polynucleotide ligase. When used in conjunction with the techniques described in this paper, the ligase will enable the preparation of radioactively labeled DNA substrates for use in identifying other enzymatic steps involved in these processes.

Summary.—Polynucleotide ligase, purified 350-fold from *E. coli* infected with T4 bacteriophage, catalyzes the covalent joining of two segments of an interrupted strand in a DNA duplex. The reaction requires ATP and results in the formation of phosphodiester bonds, AMP, and PP_i. The enzymatic repair of single-strand breaks in T7 DNA has been demonstrated by sedimentation and end-group analysis.

Note added in proof: Purified preparations of polynucleotide ligase catalyze an ATP-PP_i^{32} exchange which is not dependent on DNA. If this exchange is a property of the ligase system, then activation of the DNA may involve an enzyme-adenylate intermediate.

We acknowledge the technical assistance of Mrs. Ann Thompson.

The abbreviations used in this paper are those described in *J. Biol. Chem.* Nicked DNA is used to describe bihelical DNA containing phosphodiester bond interruptions (single-strand breaks) in each of its two strands. 5′-P^{32}-phosphoryl nicked DNA is nicked DNA containing P^{32}-labeled phosphomonoesters located both internally and externally in the DNA duplex. External and internal phosphomonoesters are used to describe the location of these groups in a DNA duplex. ATP^{32} is ATP labeled with P^{32} in the γ-phosphate.

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